Communications to the Editor

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Metabolic Pathway of L-3-Methoxy,4-hydroxyphenylalanine (3-O-MethylDOPA)—Participation of Tyrosine Aminotransferase and Lactate Dehydrogenase

Metabolic pathway of L-3-methoxy, 4-hydroxyphenylalanine (3-O-methylDOPA) in rat liver was investigated *in vitro*. 3-O-MethylDOPA was proved to be first transaminated to 3-methoxy, 4-hydroxyphenylpyruvate by tyrosine aminotransferase, which was then reduced to 3-methoxy, 4-hydroxyphenyllactate by lactate dehydrogenase. No evidence was obtained for the O-demethylation of 3-O-methylDOPA.

The 3-O-methylation of L-3,4-dihydroxyphenylalanine (L-DOPA) to 3-methoxy, 4-hydroxyphenylalanine (3-o-methylDOPA) is known as the main metabolic pathway along with the decarboxylation to dopamine.[1] Recently, Pletscher, *et al.* showed that 3-O-methylDOPA is partially demethylated to L-DOPA after intraperitoneal administration to rats and suggested the possibility of 3-O-methylDOPA as a precursor of dopamine in the brain.[2] The use of peripheral L-DOPA decarboxylase inhibitor, L-α-methyl, α-hydrazino, 3,4-dihydroxyphenylpropionic acid (MK-486), to economize the massive dose of L-DOPA, is known to increase the urinary excretion of 3-methoxy, 4-hydroxyphenyllactate (MHPL).[3] We wish to report in this communication that, in rat liver, 3-O-methylDOPA is first transaminated to 3-methoxy, 4-hydroxyphenylpyruvate (MHPP) with tyrosine aminotransferase and then reduced to MHPL with lactate dehydrogenase and that no evidence was obtained for the O-demethylation of 3-O-methylDOPA.

L-3-O-MethylDOPA-^{14}C (U) (specific activity: 337.5 mCi/mmole) was purchased from New England Nuclear Corporation. L-3-O-MethylDOPA and purified beef heart lactate dehydrogenase were obtained from Sigma Chemical Co., Ltd. The activity of 3-O-methylDOPA transaminase was measured as follows. The rat organ homogenates (0.5 ml) were incubated with 0.2 ml of pyridoxal-5′-phosphate (0.1 mM), 0.2 ml of sodium diethyldithiocarbamate (1 mM) and 0.6 ml of 3-O-methylDOPA-^{14}C (1 mM) for 5 min at 37°. α-Ketoglutarate (0.5 ml, 5 mM) was then added to the mixture and incubated for 20 min. After the termination of the reaction with the addition of 0.2 ml of 4 N–H_2SO_4, MHPP was extracted with 3 ml of ethylacetate. The radioactivity of the organic phase was measured in a Packard Liquid Scintillation Counter Model 3380. The activity of lactate dehydrogenase was measured as follows. The substrate solution (2.9 ml, 1 mM) and 0.1 ml of NADH (0.1 mM) in the cuvette were added with 0.1 ml of purified lactate dehydrogenase (30 μg). The decrease of optical density (OD) at 340 mμ was measured in a Shimazu Multipurpose Spectrophotometer. The mixture without lactate dehydrogenase was used as a reference. Potassium phosphate buffer (0.05 M, pH 7) was used in all the experiments.

As shown in Fig. 1, the liver showed the highest activity of 3-O-methylDOPA transaminase and was followed by the kidney and the heart. The other organs have no appreciable activity and the liver homogenates without addition of α-ketoglutarate also show no activity. The induction with hydrocortisone (4 hr after 50 mg/kg *i.p.* administration of hydrocortisone

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2) G. Bartholini, I. Kuruma, and A. Pletscher, *Nature*, **230**, 533 (1971); I. Kuruma, G. Bartholini, R. Tissot, and A. Pletscher, *Clin. Pharmacol. Ther.*, **12**, 678 (1971).
3) M. Sandler, R.D. Johnson, C.R.J. Ruthven, J.L. Reid, and D.B. Calne, *Nature*, **247**, 364 (1974).

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acetate) was observed only in the liver (2.48 fold) and these characteristics along with the tissue distribution of the activity are the same as reported with tyrosine aminotransferase.[4] Furthermore, the transamination of 3-O-methylDOPA was inhibited competitively with tyrosine. These results demonstrate that 3-O-methyl DOPA is metabolized to MHPP by liver tyrosine aminotransferase. Subsequently, it was shown that the substrate specificity of lactate dehydrogenase is not so high and MHPP is also a substrate of the enzyme. As shown in Table I and II, MHPP and other aromatic α-keto acids were the substrate of the enzyme and the addition

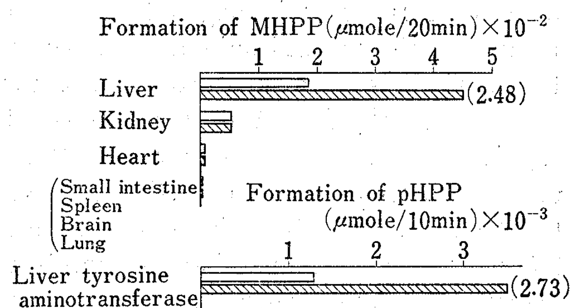

Fig. 1. Organ Distribution and Induction by Hydrocortisone of 3-O-Methyl DOPA Transaminase in Rat

□ : control
▨ : hydrocortisone induced

Table I. Reduction of MHPP with Lactate Dehydrogenase

	Activity (nmole NADH/min)
Complete system[a)]	99.7
−NADH	nil.
−Enzyme	nil.
−NADH+NADPH	nil.
−Substrate (MHPP)	nil.

a) MHPP: 1 mM, NADH: 0.1 mM, LDH: 30 μg

Table II. K_m Values of Various Aromatic α-Keto Acids to Lactate Dehydrogenase

α-Keto acid	K_m (mM)
Indolylpyruvic acid	0.5
4-Hydroxyphenylpyruvic acid	1.0
Phenylpyruvic acid	1.6
3,4-Dihydroxyphenylpyruvic acid	1.9
3-Methoxy, 4-hydroxyphenylpyruvic acid	2.7

of NADPH instead of NADH showed no activity. The same results were obtained qualitatively with dialyzed rat liver homogenates. The spot of MHPL was recognized on thin-layer chromatogram (TLC) when MHPP and NADH were incubated with dialyzed rat liver homogenates (solvent system; chloroform–ethanol–glacial acetic acid (15:5:1), Silica gel F-254, 0.25 mm of thickness, Merck). The addition of NADPH showed no activity also in the experi-

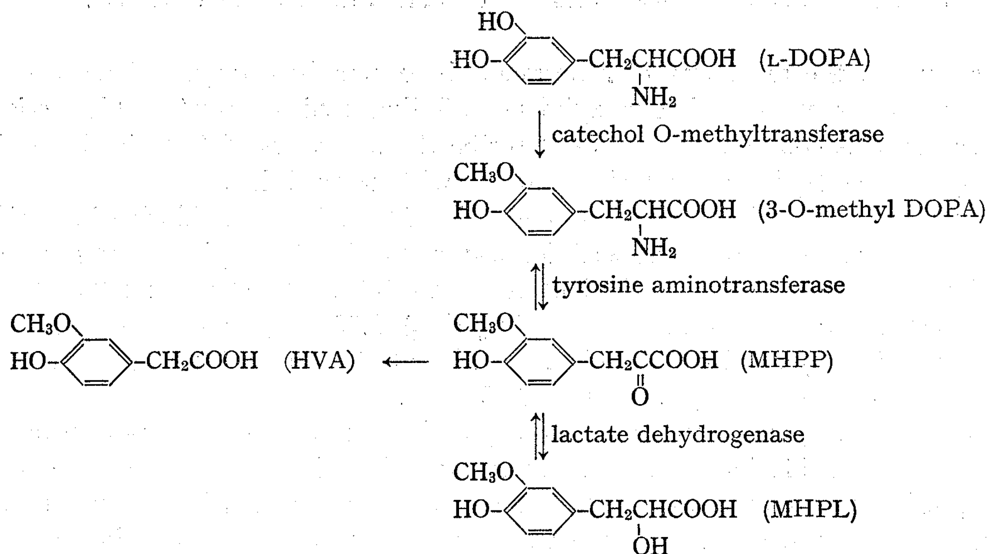

Chart 1. Metabolic Pathway of 3-O-Methyl DOPA in Rat Liver

4) E.C.C. Lin and W.E. Knox, *J. Biol. Chem.*, 233, 1186 (1958); R.J. Wurtman and F. Larin, *Biochem. Pharmacol.*, 17, 817 (1968).

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ment with rat liver homogenates. The formation of MHPL from MHPP was inhibited by pyruvate, the natural substrate of lactate dehydrogenase, but not influenced by α-ketoglutarate which is not a substrate of the enzyme. These results demonstrate that MHPP formed from 3-O-methylDOPA is then reduced to MHPL by liver lactate dehydrogenase. We recognized a small amount of homovanillate (HVA) on TLC along with MHPL suggesting an oxidative decarboxylation of MHPP to HVA. Furthermore, in isolated rat perfused liver, we have found that MK-486 increases the formation of 3-O-methylDOPA from L-DOPA and that 3-O-methylDOPA is metabolized mainly to MHPP, MHPL, and HVA, while no L-DOPA was detected.[5] Consequently, we have concluded that 3-O-methylDOPA is first transaminated to MHPP with liver tyrosine aminotransferase and then MHPP is reduced to MHPL with liver lactate dehydrogenase along with the concurrent oxidative decarboxylation of MHPP to HVA (chart 1). A more detailed study is now under investigation in our laboratory.

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5) K. Hattori, K. Tanaka, T. Maeda, and H. Shindo, the 18th Local Meeting of Pharmaceutical Soc. Japan, Tokyo, November 1974.

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Reaction of 4-(*o*-Nitrobenzylidene)-3,5-dimethylisopyrazole with Acyl Chlorides

4-(*o*-Nitrobenzylidene)-3,5-dimethylisopyrazole (**1**) was converted to 1-acyl-4-(α-hydroxy-*o*-nitrobenzyl)-3,5-dimethylpyrazoles (**2**, **3**) or 3-(1'-acyl-3',5'-dimethylpyrazolo)-5-chloroanthranils (**6**, **7**) by treatment with acyl chlorides with or without pyridine.

The literature on the reactivity of isopyrazole is extremely scanty, and recently we reported[1] some reactions of 4-(*m*-nitrobenzylidene)-3,5-dimethylisopyrazole, which exists in a betaine form.[2] In this communication, we describe the behaviors of 4-(*o*-nitrobenzylidene)-3,5-dimethylisopyrazole (**1**) in acyl chlorides with or without pyridine.

The reaction of **1** with slight excess of acetyl chloride or benzoyl chloride in pyridine at 50° for 20 hours followed by treatment with ice water afforded 1-acetyl-4-(α-hydroxy-*o*-nitrobenzyl)-3,5-dimethylpyrazole (**2**) in 72% yield, mp 152—153°, IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (CO), NMR (DMSO-d_6) δ: 1.96 and 2.43 (C_3– and C_5–C$\underline{H}_3$), 2.75 (O$\underline{H}$), 2.62 (COC$\underline{H}_3$), 6.45 (C$\underline{H}$) or 1-benzoyl derivative (**3**) as oil in 55% yield, IR $\nu_{max}^{CHCl_3}$: 3650 (OH), 1700 (CO), NMR ($CDCl_3$) δ: 1.95 and 2.50 (C_3– and C_5–C$\underline{H}_3$), 3.32 (O$\underline{H}$), 6.45 (C$\underline{H}$).

The introduction of hydroxy group at benzyl position can be explained by postulating the formation of pyridinium chloride (**4**), as reaction intermediate, because 1-acetyl-4-(α-chloro-*m*-nitrobenzyl)-3,5-dimethylpyrazole (**5**)[3] is stable against the treatment with water at room temperature. In contrast, treating a solution of **1** in acetyl chloride without pyridine at 50° for 10

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2) T. Kurihara, M. Sugiyama, H. Hirano, K. Tomita, and T. Sakaki, *J. Heterocyclic Chem.*, **12**, 541 (1975).
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